

The University of Chicago

THE ACID HYDROLYSIS
OF PROTEINS IN THE PRESENCE
OF SALTS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PHARMACOLOGY

1933

By
ABRAHAM AVROME BASS

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THE ACID HYDROLYSIS OF PROTEINS IN
THE PRESENCE OF SALTS

The object of this work is to determine the effect of salts on the hydrolysis of proteins by acid. The suggestion for carrying this out arose from the experiments of Staker and Gortner,¹ who demonstrated the existence of a definite Hofmeister series for the peptizing action of certain M/2 salt solutions on the proteins of many seeds. Generally, though not invariably, for halides Cl is less effective than Br, and Br less than I. On the other hand, Rotha and Saunders² and Saunders³ have shown that the peptizing action of certain salts shows no lyotropic series with some seeds, the amount of peptization being the same with all the neutral salts studied.

In considering the possible effects of salts on the acid hydrolysis of proteins, several factors must be taken into account. If alkali halides have a progressive peptizing action they might be expected to catalyze protein hydrolysis in the same manner. Bancroft and Richter⁴ state that whereas bromides and iodides are peptizing agents toward colloids in alkaline solution, in acid solution these ions are coagulating agents. In such case

¹E. V. Staker and R. A. Gortner, J. Phys. Chem., 35 (1931), 1565.

²L. K. Rotha and F. Saunders, J.A.C.S., 54 (1932), 342.

³F. Saunders, J.A.C.S., 53 (1931), 696.

⁴W. D. Bancroft and G. H. Richter, J. Phys. Chem., 35 (1931), 1606.

bromides and iodides would probably retard the acid hydrolysis of proteins. In either event, whether peptization or coagulation occurs, abnormalities in the hydrolysis rate are to be expected, provided that no other factors must be considered. It is well known that neutral salts increase the activity coefficients of strong acids, and such action should invariably increase the speed of hydrolysis.

Northrop⁵ hydrolyzed gelatin with 1N HCl at 65° in the presence of NaCl, KCl, and CaCl₂ ranging in concentration from 0.5N to 1.65N. In the lower concentrations the salts increased the reaction velocity to about the same extent that they increased the activity of the acid. In higher concentrations the activity increase was greater than the acceleration of the hydrolysis rate. With 0.02N HCl, KCl had practically no effect on either the activity or on the velocity of the hydrolysis.

When an attempt was made to carry out this work on seed meals several difficulties appeared at once. The meals used in the hydrolysis were extracted by salt solutions of the proper concentration, and then HCl was added. However, since the different salt solutions do not cause the same amount of peptization, it was necessary to analyze the suspensions for nitrogen and then assume that all of it is protein nitrogen. When the peptized suspension was added to the acid a heavy coagulum formed at once which did not disperse until subjected to several hours heating; thus it was not possible to determine the NH₂-N before the hydrolysis. Finally, since HCl has little peptizing action, it was impossible to have a salt-free control with which to compare the remaining samples. It was therefore felt best to use

⁵Northrop, J. Gen. Physiol., 3 (1920-1921), 715.

extracted, denatured proteins in this work, one of the advantages being that it is fairly certain that nothing but protein was present in addition to the acid-salt solution. There is, of course, the question as to whether facts obtained concerning seed meals can be compared with those obtained by working with extracted proteins. However, Osborne and Harris⁶ have shown that extracted, crystalline edestin is peptized by salts according to a Hofmeister series.

The seeds selected were hemp, sunflower, peanut, Brazil nut, almond, and squash. The first three have been shown by Staker and Gortner to give a lyotropic series, Cl less than Br less than I. The Brazil nut shows very little change in peptization with change in anion, the peanut and sunflower somewhat more, while the hemp seed gives considerable change. Potassium was used as the negative ion. The last two of the six seeds were not used by the above authors. The proteins were extracted from the seeds by a method that has been described in a previous paper.⁷

The procedure followed for the comparison of the hydrolysis rates of these vegetable proteins is the same throughout this entire work except that the salt concentrations are not always 0.5N. The following method was used.

Either 1 g. or 0.5 g. of protein was added to 100 cc. or 50 cc. respectively of the acid or acid-salt solution in a 200 cc. round bottom flask. The concentrations shown are the final ones after dilution. The same 1N HCl was used during this entire series of hydrolyses. It is clear that these are not

⁶T. B. Osborne and I. F. Harris, Ann. J. Physiol., 14 (1905), 151.

⁷Saunders, loc. cit.

strictly 1% protein solutions, but the ratio of protein to acid is always the same. The flasks were connected to reflux condensers by inner tubes with ground glass joints, and heated for controlled time intervals. After each period of heating the solutions were made up to the original weight by adding distilled water, and all sampling was likewise done by weight. The usual procedure, except in the cases when 20% HCl was used, was to remove 5cc. with a pipette, wash the sample into a 10cc. volumetric flask and dilute to volume. Two cc. were then taken for analysis for amino nitrogen by the Van Slyke volumetric method. The weight of sample removed was determined accurately. In those instances in which 20% HCl was used, 3 grams of protein were hydrolyzed with 50 cc. of acid, 2 cc. of this diluted sample being used for analysis. As before, the samples were weighed accurately.

Since these hydrolyses were carried out in open condensers there was an opportunity for concentration to take place due to loss of water. However, it was found that the loss during any one period of heating amounted to 0.00-0.50 grams as a rule and in a few instances was as much as 1.50 grams. As will be shown later this higher figure will cause at most a difference of 0.05% $\text{NH}_2\text{-N}$.

All of the flasks were heated over a water bath, and in the case of the seed proteins the entire run of any one protein was made at the same time in the same water bath. The procedure was to heat at 80° for three hours with intermittent shaking, thereafter at the boiling point of the water. There was no further shaking or stirring of the solution after the first three hours. The seed proteins were not completely in solution as a rule until after 20 hours heating, but the amount of undissolved material was very small and since duplicates checked quite well,

it would seem that the results are reliable.

The acid used was 1.073N HCl and in the experiments with the seed globulins the salt concentration was half-molar in every case. Where the hydrolyzing acid is stated to be 1N, it is understood that 1.073N is meant. The results are expressed in percent of $\text{NH}_2\text{-N}$ by weight of the original protein. The hours refer to the total time of heating. The results of the hydrolyses of the seed proteins are given below.

TABLE I
HYDROLYSIS OF SEED PROTEINS

(1) Hydrolysis of Brazil Nut Protein

Solution	Hours of heating			
	8.0	18.0	26.0	34.0
HCl + O	4.95%	7.60%	9.25%	9.45%
HCl + LiCl	5.35	8.10	9.35	9.70
HCl + LiBr	5.80	8.65	9.55	9.90
HCl + NaCl	5.50	8.30	9.35	9.85
HCl + NaBr	5.65	8.60	9.60	9.65
HCl + NaI	5.40	8.10	9.50	9.80
HCl + KCl	4.85	8.10	9.20	9.85
HCl + KBr	5.00	8.10	9.10	9.60
HCl + KI	4.95	8.00	9.05	9.60

(2) Hydrolysis of Almond Seed Protein

Solution	Hours of heating			
	8.0	18.0	26.0	34.0
HCl + O	6.25%	8.70%	9.75%	10.10%
HCl + O	6.25	8.80	9.80	10.05
HCl + LiCl	6.70	9.05	9.90	10.40
HCl + LiBr	6.55	9.05	9.80	10.30
HCl + NaCl	6.55	8.85	9.75	10.25
HCl + NaBr	6.95	9.25	9.90	10.30
HCl + NaI	5.75	8.45	9.30	9.85
HCl + KBr	5.85	8.70	9.45	10.00
HCl + KI	5.80	8.65	9.45	10.10

TABLE I (Continued)

(3) Hydrolysis of Hemp Seed Protein

Solution	Hours of heating			
	8.0	18.0	26.0	34.0
HCl + O	6.00%	8.25%	8.95%	9.85%
HCl + LiCl	6.50	8.40	9.00	9.85
HCl + LiBr	6.65	8.40	9.20	9.90
HCl + NaCl	6.40	8.25	8.95	9.85
HCl + NaBr	6.40	8.30	9.30	9.90
HCl + NaI	6.20	8.20	8.95	9.85
HCl + KCl	6.15	8.25	8.95	9.85
HCl + KBr	6.30	8.20	9.15	9.95
HCl + KI	6.15	8.30	9.10	9.75

(4) Hydrolysis of Peanut Seed Protein

Solution	Hours of heating			
	8.0	18.0	26.0	34.0
HCl + O	5.65%	8.85%	9.20%	9.75%
HCl + O	5.85	8.75	9.20	9.60
HCl + LiCl	6.10	8.85	9.60	9.70
HCl + LiBr	5.85	8.75	9.40	9.60
HCl + NaCl	5.95	8.85	9.20	9.60
HCl + NaBr	6.15	8.85	9.15	9.65
HCl + NaI	5.45	8.40	9.00	9.45
HCl + KCl	5.80	8.85	9.15	9.55
HCl + KBr	5.75	8.80	9.15	9.55

(5) Hydrolysis of Sunflower Seed Protein

Solution	Hours of heating			
	8.0	16.0	26.0	34.0
HCl + O	5.65%	8.00%	8.95%	9.45%
HCl + LiCl	6.00	8.50	9.30	9.70
HCl + LiBr	6.25	8.65	9.50	9.50
HCl + NaCl	6.00	8.50	9.60	9.60
HCl + NaBr	6.35	8.35	9.55	9.65
HCl + NaI	5.65	8.10	9.30	9.45
HCl + KCl	5.85	8.15	9.15	9.60
HCl + KBr	6.00	8.40	9.25	9.45
HCl + KI	5.70	8.00	9.25	9.30

TABLE I (Continued)

(6) Hydrolysis of Squash Seed Protein

Solution	Hours of heating			
	8.0	18.0	26.0	34.0
HCl + 0	4.60%	8.10%	8.85%	9.60%
HCl + 0	4.70	8.15	8.95	9.65
HCl + LiCl	5.15	8.30	9.15	10.00
HCl + LiBr	4.95	8.35	9.15	10.00
HCl + NaCl	5.10	8.30	9.20	9.75
HCl + NaBr	5.10	8.15	8.95	9.65
HCl + NaI	4.60	7.85	9.10	9.40
HCl + KBr	4.90	7.90	9.10	9.30
HCl + KI	4.75	7.85	8.90	9.55

Complete hydrolysis for this protein = 12.20%.

From these figures it can be seen that the hydrolysis rates of the proteins are increased in the early stages in all cases in which lithium and sodium salts were present. NaI is an exception, its effect being usually either slight or negative. The potassium salts in general are less effective. Since the catalysis is probably due to the increase in the activity coefficient of the acid by the salt, the hydrogen ion activity of the acid in the presence of several salts was measured by the hydrogen electrode.

TABLE II

a_H OF HCl IN PRESENCE OF SALTS

Solution	pH	a_H
1N HCl + 0	+0.02	0.96
1N HCl + 0.5N NaCl	- .07	1.18
1N HCl + .5N NaBr	- .05	1.12
1N HCl + .5N KCl	- .02	1.05
1N HCl + .5N KBr	- .02	1.05
1N HCl + .5N KI	- .08	1.20

KCl and KBr cause less increase in the A_H than do the

sodium salts. The KI solution was faintly yellow with free iodine, and the more positive potential obtained in this case may have been due in part to failure to realize equilibrium with the hydrogen electrode. Northrop⁸ has made measurements on the effect of 0.5N NaCl and KCl on the activity of normal HCl with which the foregoing agree.

When attempts were made to use higher concentrations of salts large quantities of undissolved protein were still present in the flasks after twenty hours heating. As it did not seem possible to obtain accurate samples the experiments were discontinued. A similar experiment with purified casein gave like results.

It then became necessary to use a readily soluble protein as it was desired, not only to use high concentrations of salts, but also to obtain the hydrogen ion activity of the solutions before hydrolysis in order to correlate increased rate of hydrolysis with increased activity. Accordingly, a sample of Eastman's gelatin was used. This gelatin had a moisture content of 10.7%, ash of 0.03% on a moisture-free basis, and its pH in 1% solution was 4.86.

Before using salt solutions with the protein it was desired to know whether 1N HCl is able to cause its complete hydrolysis. The gelatin was therefore boiled over hot plates with 20% and 1N HCl until the $\text{NH}_2\text{-N}$ became constant.

⁸J. Northrop, loc. cit.

TABLE III
HYDROLYSIS OF GELATIN BY 20% AND 1N HCl

	20% HCl			1N HCl		
	Time of heating--in hours					
	24.0	48.0	72.0	100.0	125.0	150.0
Percent NH ₂ -N	11.50	11.70	11.45	11.60	11.95	11.90

Although considerably more time is necessary, 1N HCl is able to cause complete hydrolysis and in fact the highest figure is a little above that for 20% HCl. This is probably due to greater decomposition of the protein, with humus formation, in the case of the stronger acid. None of the values given here or subsequently for gelatin are corrected to a moisture-free basis.

The first experiment on the effect of salts on the rate of hydrolysis of gelatin was carried out with NaCl in varying concentrations as indicated, using 0.5 g. of gelatin and 50 cc. of the acid-salt solutions. The time intervals refer to hours of heating over a boiling water bath. There was no preliminary three hours heating at 80 .

TABLE IV
HYDROLYSIS OF GELATIN IN PRESENCE OF HCl

Solution	Time of heating--in hours				
	5.0	10.0	20.0	30.0	40.0
	Percent $\text{NH}_2\text{-N}$				
1N HCl + 0	7.05%	8.05%	9.05%	9.70%	10.10%
1N HCl + 0.5N NaCl	7.40	8.40	9.45	9.85	10.20
1N HCl + 2N NaCl	7.60	8.85	9.95	10.30	10.30
1N HCl + 3N NaCl	8.05	9.10	10.00	10.30	10.40
1N HCl + 4N NaCl	8.15	9.20	10.20	10.45	10.60
Complete hydrolysis	= 11.95%				
Gelatin blank	= 0.55%				

In the following table is shown the effect of NaCl in various concentrations on the hydrogen ion activity of N HCl in the absence of gelatin. All measurements were made by the hydrogen electrode at room temperature.

TABLE V
EFFECT OF NaCl ON a_H OF N HCl

Solution	pH	a_H	Percent Increase
1N HCl	+0.06	0.87	-
1N HCl + .5N NaCl	- .03	1.07	23
1N HCl + 1N NaCl	- .13	1.34	54
1N HCl + 2N NaCl	- .30	2.00	130
1N HCl + 3N NaCl	- .45	2.82	224
1N HCl + 4N NaCl	- .68	4.78	451

In order to correlate as well as possible the increase in hydrogen ion activity with the increase in hydrolysis rate, the velocity constants have been calculated according to a reaction of the first order. It is to be emphasized that this hydrolysis is known not to be of this order, and it is doubtful whether it can be assigned to any particular reaction order because of the complexity of the breakdown of the protein. However, since the amount of hydrolysis is proportional to the concentration of unhydrolyzed protein, it is necessary to use some type of calculation which takes this into account, and the monomolecular equation probably comes as close to giving the true velocity constants as does any other equation.

When the constants were calculated for fixed time intervals in the usual manner it was observed that the values dropped steadily with increasing time. This shows that the "average order of the reaction" is greater than the first order. The velocity constants were therefore compared at the same stage

of hydrolysis, the time intervals being determined by picking off the points on curves drawn from the analytical data. In several instances the curves were extrapolated for very short intervals in order to obtain desired points.

The percentage of the total hydrolysis at the time intervals when samples were taken was determined from the figures for $\text{NH}_2\text{-N}$, the time intervals corresponding to the same stage of hydrolysis for all the samples was then calculated as just described, the velocity constants and percent change in the constants were determined from these values. The results are given in the tables below. In all this work allowance has been made for 0.55% $\text{NH}_2\text{-N}$ in the gelatin before hydrolysis.

TABLE VI

HYDROLYSIS OF GELATIN IN PRESENCE OF NaCl (1)

(1) Percent completion of hydrolysis at fixed time intervals

Solution	Time of heating--in hours				
	5.0	10.0	20.0	30.0	40.0
1N HCl + 0	56.8	65.5	74.3	80.0	83.5
1N HCl + 0.5N NaCl	59.8	68.6	77.7	81.2	84.3
1N HCl + 2N NaCl	61.6	72.5	82.1	85.2	85.2
1N HCl + 3N NaCl	65.6	74.6	82.5	85.2	86.0
1N HCl + 4N NaCl	66.3	75.6	84.2	86.5	87.8

(2) Time intervals for fixed stages of hydrolysis

Solution	Percent completion of hydrolysis			
	65	70	75	80
1N HCl + 0	582	816	1158	1776
1N HCl + 0.5N NaCl	450	672	978	1458
1N HCl + 2N NaCl	366	510	708	1114
1N HCl + 3N NaCl	288	432	624	942
1N HCl + 4N NaCl	276	408	576	852

TABLE VI (Continued)

(3) Velocity constants for fixed stages of hydrolysis

Solution	Percent completion of hydrolysis			
	65	70	75	80
	$K \times 10^2$			
1N HCl + 0	0.180	0.147	0.119	0.039
1N HCl + 0.5N NaCl	.233	.179	.142	.047
1N HCl + 2N NaCl	.286	.236	.196	.068
1N HCl + 3N NaCl	.364	.279	.222	.073
1N HCl + 4N NaCl	.380	.295	.241	.081

Solution	Percent increase in K				Average
1N HCl + 0.5N NaCl	29.5	21.8	19.3	20.5	22.8
1N HCl + 2N NaCl	59.0	60.5	64.7	74.4	64.7
1N HCl + 3N NaCl	102.0	89.9	86.5	87.3	91.4
1N HCl + 4N NaCl	111.0	100.5	102.5	107.8	105.2

The velocity constant, K, was calculated from the formula $K = 2.303/t \times \log 1/1-z$ where z is the percent hydrolyzed at the time t, which is expressed in minutes. The increases in K are referred to 1N HCl as a basis.

The large variations in the increases are due partly to the large experimental error and partly to the fact that the course of the hydrolysis itself is not perfectly regular. An attempt to correlate increased hydrolysis rate with increased hydrogen ion activity of the acid as shown in a previous table demonstrated that, except in the case of 0.5N NaCl, the activity increase is always greater than the hydrolysis increase.

In order to learn whether this is also true in the early stages of the breakdown of the protein, the above-mentioned hydrolysis was repeated in more detail. A solution of 2N HCl was used in place of 1N HCl + 2N NaCl. The a_H of this 2N HCl was 138% greater than that of 1N HCl. The results are tabulated below in the same manner as that given previously.

TABLE VII
HYDROLYSIS OF GELATIN IN THE PRESENCE
OF NaCl (2)

(1) Percent $\text{NH}_2\text{-N}$ at fixed time intervals					
Time-- in hours	1N HCl	1N HCl + 0.5N NaCl	1N HCl + 3N NaCl	1N HCl + 4N NaCl	2N HCl
0.20	1.90%	2.10%	2.30%	2.65%	3.00%
.60	3.25	3.50	4.30	4.55	5.40
1.80	5.35	5.55	6.40	6.85	7.15
3.4	6.60	6.70	7.45	7.90	8.10
5.0	7.40	7.90	8.60	8.80	8.95
10.0	8.65	9.05	9.75	9.90	10.15
20.0	9.75	10.25	10.45	10.85	11.15
30.0	10.40	10.80	11.00	11.00	11.30
40.0	10.75	10.80	11.00	11.20	11.25
Complete hydrolysis = 11.85%					
Gelatin blank = 0.55%					

(2) Percent completion of hydrolysis at fixed time intervals

0.20	11.9%	13.6%	15.4%	18.5%	21.6%
.60	23.8	26.0	33.0	35.2	42.7
1.80	42.3	44.1	51.6	55.5	58.2
3.4	53.3	54.2	60.8	64.8	66.5
5.0	60.3	64.8	70.9	72.7	74.0
10.0	71.4	74.9	81.1	82.4	84.6
20.0	81.1	85.5	87.2	90.8	93.4
30.0	86.8	90.4	92.2	92.4	94.7
40.0	90.1	90.4	92.2	93.9	94.4

(3) Time intervals for fixed stages of hydrolysis

Percent hydro- lysis	Time--in minutes				
25.0	41.0	33.0	24.0	21.0	15.0
30.0	52	47	32	29	22
40.0	99	90	55	48	33
50.0	177	153	102	79	63
60.0	303	270	183	144	126
70.0	546	441	321	261	237
80.0	1092	882	561	477	426
90.0	2400	1692	1530	1080	912

TABLE VII (Continued)

(4) Velocity constants for fixed stages of hydrolysis

Percent Hydrolysis	1N HCl	1N HCl + 0.5N NaCl	1N HCl + 3N NaCl	1N HCl + 4N NaCl	2N HCl
	K x 10 ²				
25	0.672	0.928	1.200	1.370	1.918
30	.603	.673	.990	1.093	1.458
40	.516	.567	.936	1.065	1.548
50	.391	.452	.679	.874	1.099
60	.303	.340	.502	.638	.729
70	.220	.273	.375	.461	.508
80	.131	.162	.255	.300	.335
90	.096	.136	.157	.213	.252

(5) Percent increase in K

25	-	39.1	78.6	103.8	185.5
30	-	11.6	64.3	81.3	141.8
40	-	10.1	81.4	106.2	200.0
50	-	15.6	73.7	123.4	181.1
60	-	12.2	65.7	110.5	140.5
70	-	24.1	70.6	109.6	130.9
80	-	23.6	94.8	123.9	155.6
90	-	41.7	63.5	123.0	162.6
Average increase		22.3	74.1	110.2	162.5

From the foregoing figures it can be seen that, although at 25° 2N HCl has a much lower a_H than 1N HCl containing either 3N or 4N NaCl, (as shown on pages 10 and 12), yet at 100° its rate of hydrolysis is more rapid than that of either.

There are two explanations that might be advanced to account for the observed discrepancies between increase in hydrolysis rate and increase in activity. First, the activities were measured in the absence of gelatin, which may have a varying ability to bind acid in the presence of the salt. Second, the activities were all measured at 25° and the hydrolyses were carried out at 100°; the acid-salt solutions may have a lower temperature coefficient of a_H than the acid alone.

In order to demonstrate the first point, the a_H was

determined of N HCl and N HCl-NaCl solutions in the absence and in the presence of 1% gelatin, the concentrations of acid and salt being the final ones after all dilutions had been made. The acid concentration was the same in all cases.

TABLE VIII
a_H OF HCl AND HCl-NaCl IN PRESENCE AND
ABSENCE OF GELATIN

Solution	No gelatin		1% gelatin	
	pH	a _H	pH	a _H
1N HCl + 0	+0.06	0.87	+0.06	0.87
1N HCl + .5N NaCl	-0.03	1.07	-0.03	1.07
1N HCl + 1N NaCl	-0.13	1.34	-0.11	1.28

It is apparent that 1% gelatin solutions have no appreciable acid binding power in the presence of 1N HCl alone or with 0.5N NaCl added, but that gelatin in the presence of 1N NaCl causes a slight rise in the pH. 2N NaCl precipitated the gelatin. However, if the acid-binding power of the protein and its split products is very slight under these conditions, it should be possible to measure the E.M.F. after the hydrolysis and obtain the desired information. It has already been demonstrated that heating the acid solution containing gelatin will cause the protein to dissolve no matter how high the concentration of salt. The pH of solutions of 2N HCl and of 3N NaCl in 1N HCl was determined in the absence of gelatin, 0.5 g. samples of the protein were hydrolyzed with 50 cc. portions of these solutions, analyzed after 8 hours and after 20 hours, and the pH determined after the second period of heating.

TABLE IX
 a_H OF HCl AND HCl-NaCl IN PRESENCE AND
 ABSENCE OF GELATIN

Solution	Percent $\text{NH}_2\text{-N}$			
	8.0 hours		20.0 hours	
1N HCl + 3N NaCl	9.15%		10.45%	
" "	8.95		10.45	
2N HCl	9.60		11.25	
" "	9.70		11.25	
Solution	Before hydrolysis (no gelatin present)		After hydrolysis of gelatin	
	pH	a_H	pH	a_H
1N HCl + 3N NaCl	-0.52	3.31	-0.48	3.02
2N HCl	-0.36	2.29	-0.35	2.24

This experiment shows that the pH of neither the acid nor acid-salt solution is affected very much by the gelatin and that the difference in the rates of hydrolysis cannot be so explained. (This assumes that the binding power of the gelatin would be no greater at 100° than at 25°, at which temperature the activities were measured). The hydrolysis by 2N HCl is approximately 95% complete after 20 hours and not much more change in pH is to be expected after that. Strictly speaking, these results are not comparable as in one case the acid solution has been diluted with a volume corresponding to 0.5 g. of gelatin in 50 cc. Since the dilution and the binding of acid should both operate in the direction of making the pH more alkaline, the very slight changes actually observed are good evidence that this dilution factor is negligible.

As the acid-binding power of the gelatin did not seem to be responsible for observed discrepancies between increase

in hydrolysis rate and increase in a_H , the a_H of 1N HCl and 1N HCl containing 3N and 4N NaCl was determined at 25°, 36.5°, and 47°. It was found impracticable to work at higher temperatures. The E.M.F. of N/10 HCl was also measured at the same time under the same conditions. The N/10 HCl was assumed to have the same pH, 1.076, at all temperatures, and the pH of the other solutions was calculated from this.

The values at 25° and 36.5° were determined in constant temperature rooms, the value for 47° in an oven. In all cases, the temperature was read with a thermometer immersed in the liquid. The method used was to bubble the hydrogen through a bottle of the same solution being read and then into the electrode vessel. For the pH calculation the pressure of the hydrogen was corrected for the vapor pressure of water at the temperature in question. The results are given in the following table.

TABLE X
TEMPERATURE COEFFICIENTS OF a_H OF
HCl AND HCl-NaCl

Solution	Temperature					
	25.0		36.5		47.0	
	a_H	% increase in a_H	a_H	% increase in a_H	a_H	% increase in a_H
1N HCl + 0	0.89	-	0.95	-	1.29	-
1N HCl + 3N NaCl	3.23	263	3.09	225	3.16	145
1N HCl + 4N NaCl	4.57	413	4.78	403	5.12	288

The increase in a_H is referred to 1N HCl as a basis. Although the activity at 47° is certainly not that at 100°, yet the trend of the curve appears to be shown well enough. The increase in the a_H of 1N HCl is considerably greater than that of

the acid-salt solution, and it seems likely that the apparent discrepancies in hydrolysis increase as compared with activity increase are only an expression of varying temperature coefficients of a_H . A comparison of increase in hydrolysis rate with increase in activity at 47° for 3N and 4N NaCl (in the presence of 1N HCl) shows that although there is still a gap between the two, it is much smaller than is the case when the comparison is made with activities determined at 25°.

KBr in different concentrations was next used as the catalytic agent in the hydrolysis of gelatin. The results are tabulated below in the previous manner.

TABLE XI
HYDROLYSIS OF GELATIN IN PRESENCE OF KBr

(1) Percent of NH_2 -N at fixed time intervals					
Time-- in hours	1N HCl	1N HCl + 0.5N KBr	1N HCl + 1N KBr	1N HCl + 2N KBr	1N HCl + 3N KBr
5.0	7.40%	7.30%	7.70%	8.05%	8.20%
10.0	9.05	9.00	9.20	9.70	9.85
20.0	9.95	9.95	10.10	10.60	10.60
30.0	10.65	10.70	10.75	11.10	11.15
Complete hydrolysis = 11.95%					
Gelatin blank = 0.55%					

(2) Percent completion of hydrolysis at fixed time intervals					
5.0	59.8%	59.0%	62.4%	65.5%	66.8%
10.0	74.1	73.8	75.6	79.9	81.2
20.0	82.0	82.0	83.4	88.0	88.0
30.0	88.3	88.8	89.2	92.2	92.5

(3) Time intervals for fixed stages of hydrolysis

Percent hydrolysis	Time--in minutes				
65	444	456	348	294	282
70	492	504	444	366	336
75	630	642	576	468	432

TABLE XI (Continued)

Percent hydrolysis	1N HCl	1N HCl + 0.5N KBr	1N HCl + 1N KBr	1N HCl + 2N KBr	1N HCl + 3N KBr
	Time--in minutes				
80	888	888	804	600	552
85	1422	1356	1194	852	792

(4) Velocity constants for fixed stages of hydrolysis

Percent hydrolysis	$K \times 10^2$				
65	0.236	0.230	0.302	0.357	0.372
70	.245	.239	.271	.328	.358
75	.219	.216	.241	.296	.321
80	.182	.182	.200	.268	.291
85	.133	.140	.159	.222	.239

(5) Percent increase in K

65	-	2.5 ^a	28.0	51.3	57.7
70	-	2.5 ^a	10.6	33.8	46.1
75	-	1.4 ^a	10.0	35.1	46.6
80	-	0.0	9.9	47.3	60.0
85	-	5.3	19.6	62.0	79.8

^aDecrease

Average increase	0.2 ^a	15.6	45.3	58.0
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^aDecrease

TABLE XII

EFFECT OF KBr ON a_H OF N HCl

Solution	pH	a_H	Percent Increase
1N HCl + 0	+0.02	0.96	-
1N HCl + 0.5N KBr	-0.02	1.05	9
1N HCl + 1N KBr	-0.11	1.29	34
1N HCl + 2N KBr	-0.29	1.95	103
1N HCl + 3N KBr	-0.42	2.63	174

KBr has much less effect on the activity coefficient than does NaCl in the same concentrations, and therefore shows

less catalytic action. However, the gap between increase in hydrolysis rate and increase in activity is much greater with KBr and points to a lower temperature coefficient of activity than in the case of NaCl. The hydrogen ion activities were not determined at a temperature above 25° for this salt.

In an attempt to demonstrate a Hofmeister series for alkali halides, gelatin was hydrolyzed as before with 1N HCl in the presence of the salts indicated. 4N NaCl, which has been used previously, is included for comparison.

TABLE XIII
HYDROLYSIS OF GELATIN IN PRESENCE OF
ALKALI HALIDES

Solution	Time of heating--in hours			
	5.0	10.0	20.0	30.0
	Percent $\text{NH}_2\text{-N}$			
1N HCl + 0	7.80%	8.35%	9.50%	10.25%
1N HCl + 0	7.60	8.55	9.70	10.20
1N HCl + 4N NaCl	8.80	9.90	10.85	11.00
1N HCl + 4N NaBr	8.65	9.60	10.35	10.85
1N HCl + 4N NaI	8.20	9.35	10.10	10.75
1N HCl + 4N KBr	8.65	9.40	10.50	10.75
1N HCl + 3N KI	7.90	9.25	9.75	10.40
1N HCl + 4N LiCl	8.70	9.75	10.65	10.80
1N HCl + 3N LiBr	8.45	9.50	10.55	10.75

There is nothing which can be pointed out as definitely indicating a lyotropic series, the observed differences between the halides being small. The use of salts of such high concentrations is hardly to be expected to favor peptization, but since half-molar salt solutions had no action in this direction on the seed proteins, it was thought that perhaps an effect might be demonstrated in the opposite sense; namely, of coagulation. The increases in the rate of hydrolysis observed are to be interpreted

solely in terms of the increases in hydrogen ion activity caused by the addition of the salts, and probably neither peptization nor coagulation plays any role whatever.

It was noted that the iodide solutions always liberated free iodine during the hydrolysis and to learn whether this was responsible for the general lag of these solutions, gelatin was hydrolyzed in the presence of 0.5N NaI, both with and without the addition of 0.1M iodine. This concentration of iodine was obviously much greater than that in any of the flasks containing iodides. The figures show that the added iodine has no effect.

TABLE XIV
HYDROLYSIS OF GELATIN IN PRESENCE OF
ADDED IODINE

Solution	Time of heating--in hours			
	5.0	10.0	20.0	30.0
	Percent $\text{NH}_2\text{-N}$			
1N HCl + 0	7.30%	8.70%	9.75%	10.40%
1N HCl + 0	7.25	8.85	9.75	10.40
1N HCl + 0.5N NaI	7.40	8.55	9.75	10.05
1N HCl + 0.5N NaI + 0.1M Iodine	7.15	8.50	9.70	10.00

The chlorides of the alkaline earths were used next in various concentrations as indicated. The results are tabulated below.

TABLE XV
HYDROLYSIS OF GELATIN IN PRESENCE OF ALKALI EARTH CHLORIDES

(1) Percent $\text{NH}_2\text{-N}$ at fixed time intervals					
Solution	Time--in hours				
	1.1	5.0	10.0	20.0	30.0
1N HCl + 0	4.85%	7.65%	8.70%	9.70%	
1N HCl + 1N CaCl_2	5.30	8.25	9.20	10.45	

TABLE XV (Continued)

Solution	Time--in hours				
	1.1	5.0	10.0	20.0	30.0
1N HCl + 2N CaCl ₂	5.65	8.80	9.75	10.80	
1N HCl + 1N BaCl ₂	5.00	7.95	9.05	10.30	
1N HCl + 2N BaCl ₂	5.05	8.15	9.40	10.30	
1N HCl + 0	4.40	7.45	8.70	9.80	10.30
1N HCl + 0.5N CaCl ₂	4.50	7.45	8.75	9.75	10.25
1N HCl + 3N CaCl ₂	5.20	8.30	9.65	10.60	10.85
1N HCl + 0.5N BaCl ₂	4.50	7.50	8.70	9.90	10.40
1N HCl + 0		7.05	8.05	9.05	9.70
1N HCl + 4N CaCl ₂		8.35	9.55	10.35	10.80
1N HCl + 6N CaCl ₂		9.00	10.10	10.60	10.90
1N HCl + 0		7.30	8.70	9.75	10.40
1N HCl + 8N CaCl ₂		9.20	10.70	11.40	11.50
1N HCl + 4N MgCl ₂		8.85	10.15	10.95	11.15
Complete hydrolysis for first nine solutions				= 11.85%	
Complete hydrolysis for remaining 6 solutions				= 11.95%	
Gelatin blank				= 0.55%	

(2) Percent completion of hydrolysis at fixed time intervals

1N HCl + 0	37.8%	63.0%	72.3%	81.0%	
1N HCl + 1N CaCl ₂	41.8	67.8	76.3	87.1	
1N HCl + 2N CaCl ₂	44.9	72.6	81.1	90.4	
1N HCl + 1N BaCl ₂	39.2	65.2	74.8	86.0	
1N HCl + 2N BaCl ₂	39.6	66.9	77.9	86.0	
1N HCl + 0	34.0	60.8	71.8	81.5	86.0
1N HCl + 0.5N CaCl ₂	34.8	60.8	72.2	81.1	85.5
1N HCl + 3N CaCl ₂	41.0	68.3	80.1	88.8	90.8
1N HCl + 0.5N BaCl ₂	34.8	61.2	71.8	82.4	86.9
1N HCl + 0		56.8	65.6	74.3	80.0
1N HCl + 4N CaCl ₂		68.2	78.5	85.6	89.6
1N HCl + 6N CaCl ₂		73.9	83.5	89.7	90.5
1N HCl + 0		59.0	72.1	80.3	86.1
1N HCl + 8N CaCl ₂		75.5	88.8	94.8	95.6
1N HCl + 4N MgCl ₂		72.5	83.8	90.9	92.8

(3) Time intervals for fixed stages of hydrolysis

Solution	Percent hydrolysis				
	45	50	60	70	80
	Time--in minutes				
1N HCl + 0	114	153	258	444	1131
1N HCl + 1N CaCl ₂	89	120	199	342	684

TABLE XV (Continued)

Solution	Percent hydrolysis				
	45	50	60	70	80
	Time--in minutes				
1N HCl + 2N CaCl ₂	66	90	162	264	468
1N HCl + 1N BaCl ₂	102	138	229	396	801
1N HCl + 2N BaCl ₂	96	126	212	360	660
1N HCl + 0	130	180	286	516	972
1N HCl + 0.5N CaCl ₂	128	180	284	528	1020
1N HCl + 3N CaCl ₂	84	118	204	312	597
1N HCl + 0.5N BaCl ₂	126	177	282	498	924

(3b) Time intervals for fixed stages of hydrolysis

Solution	Percent hydrolysis		
	75	80	85
	Time--in minutes		
1N HCl + 0	1254	1800	
1N HCl + 4N CaCl ₂	441	678	
1N HCl + 6N CaCl ₂	318	483	
1N HCl + 0	723	1041	1746
1N HCl + 8N CaCl ₂	294	375	480
1N HCl + 4N MgCl ₂	348	471	675

(4a) Velocity constants for fixed stages of hydrolysis

Solution	Percent hydrolysis				
	45	50	60	70	80
	K x 10 ²				
1N HCl + 0	0.516	0.413	0.355	0.271	0.141
1N HCl + 1N CaCl ₂	.660	.527	.461	.352	.235
1N HCl + 2N CaCl ₂	.891	.703	.566	.456	.344
1N HCl + 1N BaCl ₂	.576	.458	.400	.304	.209
1N HCl + 2N BaCl ₂	.612	.502	.432	.334	.244
1N HCl + 0	.452	.352	.320	.233	.166
1N HCl + 0.5N CaCl ₂	.459	.352	.323	.228	.158
1N HCl + 3N CaCl ₂	.700	.536	.449	.386	.269
1N HCl + 0.5N BaCl ₂	.467	.357	.325	.242	.174

(4b) Velocity constants for fixed stages of hydrolysis

TABLE XV (Continued)

Solution	Percent hydrolysis		
	75	80	85
	$K \times 10^2$		
1N HCl + 0	.111	.089	
1N HCl + 4N CaCl_2	.314	.237	
1N HCl + 6N CaCl_2	.436	.333	
1N HCl + 0	.192	.155	.108
1N HCl + 8N CaCl_2	.472	.426	.395
1N HCl + 4N MgCl_2	.398	.342	.281

(5a) Percent increase in velocity constants

Solution	Percent hydrolysis					
	45	50	60	70	80	
	Percent increase					Average
1N HCl + 0.50N CaCl_2	1.6	0	0.9	- 2.2	- 3.6	- 0.7
1N HCl + 1N CaCl_2	25.2	27.6	29.8	29.8	(64.5)	28.1
1N HCl + 2N CaCl_2	72.8	70.4	59.4	68.3	(145.0)	67.7
1N HCl + 3N CaCl_2	54.9	52.4	40.4	65.8	62.1	55.1
1N HCl + 0.5N BaCl_2	3.3	1.4	1.6	3.9	4.8	3.0
1N HCl + 1N BaCl_2	11.6	10.9	12.7	12.2	(48.2)	11.9
1N HCl + 2N BaCl_2	18.6	21.6	21.7	23.2	(73.3)	21.3

(5b) Percent increase in velocity constants

Solution	Percent hydrolysis		
	75	80	85
	Percent increase		
1N HCl + 4N CaCl_2	183.0	166.0	
1N HCl + 6N CaCl_2	293.0	274.0	
1N HCl + 8N CaCl_2	198.0	175.0	266.0
1N HCl + 4N MgCl_2	107.1	120.5	160.5

TABLE XVI

EFFECT OF CaCl_2 AND BaCl_2 ON a_H OF N HCl

Solution	pH	a_H	Percent increase
1N HCl + 0	+0.02	0.96	-
1N HCl + 0.5N CaCl_2	-.06	1.15	20
1N HCl + 1N CaCl_2	- .14	1.38	44

TABLE XVI (Continued)

Solution	pH	a_H	Percent increase
1N HCl + 2N CaCl_2	-0.34	2.19	129
1N HCl + 2N CaCl_2	- .56	3.63	278
1N HCl + 0.5N BaCl_2	- .05	1.12	17
1N HCl + 1N BaCl_2	- .12	1.32	38
1N HCl + 2N BaCl_2	- .31	2.04	113
1N HCl + 0	+ .01	.98	-
1N HCl + 4N CaCl_2	- .74	5.50	461
1N HCl + 6N CaCl_2	-1.31	20.42	1994
1N HCl + 8N CaCl_2	-1.87	74.14	7465

CaCl_2 has about the same effect on the activity coefficient of the acid as does NaCl in similar concentrations. The action of BaCl_2 is regularly a little less. However, the effect of the salts on the hydrolysis rate is much different. Neither CaCl_2 nor BaCl_2 causes any particular catalysis in 0.5N solution, unlike NaCl. 1N and 2N BaCl_2 are much less effective than similar concentrations of CaCl_2 ; likewise, the difference between the hydrolysis rates for the two concentrations of BaCl_2 are smaller than might be expected from their respective activities. If the a_H at the temperature of hydrolysis is the dominant factor determining the hydrolysis rate, then BaCl_2 must have a lower temperature coefficient of a_H than does CaCl_2 .

In 1N and 2N solution, CaCl_2 has marked catalytic action in a progressive manner, but in higher concentrations the results become erratic. The fact that 3N CaCl_2 seems to have less action than does 2N may be due to some error, but the value for the 8N solution, scarcely greater than that for 4N and less than that for 6N, was obtained in two independent runs which agreed very closely. The results for the higher concentrations have not been averaged because of the few figures available. It would be desirable to repeat these determinations in order to obtain

values in earlier stages of hydrolysis. The figures enclosed in parentheses have not been included in the average because the curve became very irregular toward the end of the heating in this particular run.

The one sample of MgCl_2 was included in order to see whether this salt had effect strikingly different from that of the others. The figures show that it does not.

Despite the irregularities observed with the higher concentrations of CaCl_2 , it is clear that, just as in the cases of the other salts, there is a wide gap between increase in hydrolysis and increase in activity. Since this may well be due to a difference in temperature coefficient between acid and acid-salt solutions, any action that the salts may have on the protein is masked.

The results obtained by hydrolyzing gelatin in the presence of 1.5N CuCl_2 , FeCl_3 , and Al_2Cl_6 are tabulated below.

TABLE XVII
HYDROLYSIS OF GELATIN IN PRESENCE OF
 CuCl_2 , FeCl_3 , Al_2Cl_6

(1) Percent NH_2 -N at fixed time intervals					
Solution	Time--in hours				
	1.1	5.0	10.0	20.0	30.0
1N HCl + 0		7.00%	8.35%	9.20%	9.95%
1N HCl + 1.5N FeCl_3		7.85	8.95	10.10	10.50
1N HCl + 0	4.40	7.45	8.70	9.80	10.30
1N HCl + 1.5N CuCl_2	4.75	7.70	8.75	10.00	10.25
1N HCl + 1.5N Al_2Cl_6	5.10	7.85	9.15	9.95	10.85
(2) Percent completion of hydrolysis at fixed time intervals					
1N HCl + 0		57.3%	68.2%	75.5%	82.1%
1N HCl + FeCl_3		63.8	73.5	83.5	86.9

TABLE XVII (Continued)

Solution	Time--in hours				
	1.1	5.0	10.0	20.0	30.0
1N HCl + O	34.0	60.8	71.8	81.5	86.0
1N HCl + CuCl ₂	37.0	63.0	72.3	83.3	85.5
1N HCl + Al ₂ Cl ₆	40.1	64.3	75.8	83.0	90.9

(3) Time intervals for fixed stages of hydrolysis

Solution	Percent of Hydrolysis						
	45	50	60	65	70	75	80
	Time--in minutes						
1N HCl + O				480	702	1056	1548
1N HCl + FeCl ₃				330	480	672	942
1N HCl + O	130	180	286		516		972
1N HCl + CuCl ₂	114	156	264		462		882
1N HCl + Al ₂ Cl ₆	97	141	246		422		789

(4) Velocity constants for fixed stages of hydrolysis

Solution	Percent of hydrolysis			
	65	70	75	80
	K x 10 ²			
1N HCl + O	0.235	0.171	0.122	0.104
1N HCl + FeCl ₃	.318	.250	.206	.171
1N HCl + FeCl ₃	Percent increase in K			
	35.3	45.2	68.7	64.6
				Average
				53.5

(4a) Velocity constants for fixed stages of hydrolysis

Solution	Percent of hydrolysis				
	45	50	60	70	80
	K x 10 ²				
1N HCl + O	0.452	0.352	0.320	0.233	0.166
1N HCl + CuCl ₂	.524	.444	.347	.260	.183
1N HCl + Al ₂ Cl ₆	.616	.492	.373	.285	.204
1N HCl + CuCl ₂	Percent increase in K				
	15.9	26.1	8.5	11.6	10.2
1N HCl + Al ₂ Cl ₆	36.6	39.8	16.5	22.4	22.9
					Average
					14.7
					27.6

Since FeCl_3 and CuCl_2 are oxidizing agents, it is impossible to determine the hydrogen ion activities of their solutions with the hydrogen electrodes. The Al_2Cl_6 was found to increase the a_{H} of the acid by 94%, which is much more than its effect on the hydrolysis rate, about 27%. The greatest effect on the hydrolysis rate was shown by the iron salt, while that of the copper salt was the least. Higher concentrations than 1.5N were not used because of the excessive blanks given by FeCl_3 in the determination of $\text{NH}_2\text{-N}$.

Effect of Sulfates

The results of the hydrolysis of gelatin in the presence of various sulfates are tabulated below in the same manner as that used previously.

TABLE XVIII
HYDROLYSIS OF GELATIN IN THE PRESENCE
OF SULFATES

(1) Percent $\text{NH}_2\text{-N}$ at fixed time intervals					
Solution	Time--in hours				
	1.1	5.0	10.0	20.0	30.0
1N HCl + 0	4.85%	7.70%	8.75%	9.75%	10.40%
1N HCl + 1N Na_2SO_4	3.70	6.75	8.00	9.10	
1N HCl + 1N K_2SO_4	3.75	6.75	7.95	8.95	
1N HCl + 3N $\text{Al}_2(\text{SO}_4)_3$	2.85	6.20	7.65	8.75	9.40
1N HCl + 0	4.40	7.45	8.70	9.80	10.30
1N HCl + 1N $\text{Al}_2(\text{SO}_4)_3$	3.65	6.65			
1N HCl + 2N $\text{Al}_2(\text{SO}_4)_3$	3.20	6.45	7.80	9.15	9.70
1N HCl + 0		7.50	8.75	9.95	10.35
1N HCl + 1N MgSO_4		6.70	7.90	9.35	9.60
1N HCl + 2N MgSO_4		5.50	6.85	8.60	8.90
1N HCl + 2N Na_2SO_4		4.55	6.15	7.70	7.90
1N H_2SO_4 + 0		5.60	7.20	8.55	8.95
1N H_2SO_4 + 2N Na_2SO_4		2.40	4.05	5.50	6.15

TABLE XVIII (Continued)

Solution	Time--in hours				
	1.1	5.0	10.0	20.0	30.0
1N HCl + 0		7.30	8.80	9.75	10.40
1N HCl + 4N MgSO ₄		4.60	6.30	7.65	8.30
1N HCl + 0		7.50	9.05	9.90	10.50
1N HCl + 2N CuSO ₄		5.95	7.50	8.55	9.05
Completion of hydrolysis for first seven solutions = 11.85%					
Completion of hydrolysis for remainder = 11.95%					
Gelatin blank = 0.55%					

(2) Percent completion of hydrolysis at fixed time intervals

1N HCl + 0	37.9	63.0	72.3	81.0	86.8
1N HCl + 1N Na ₂ SO ₄	27.8	54.7	65.7	75.3	
1N HCl + 1N K ₂ SO ₄	28.2	54.7	65.3	74.0	
1N HCl + 3N Al ₂ (SO ₄) ₃	20.3	49.8	62.6	72.3	78.0
1N HCl + 0	34.0	60.8	71.8	81.5	86.0
1N HCl + 1N Al ₂ (SO ₄) ₃	27.3	53.8			
1N HCl + 2N Al ₂ (SO ₄) ₃	23.4	52.0	63.8	75.8	80.7
1N HCl + 0		60.7	71.6	82.1	85.6
1N HCl + 1N MgSO ₄		53.7	64.3	76.9	79.0
1N HCl + 2N MgSO ₄		43.3	55.0	70.4	73.0
1N HCl + 2N Na ₂ SO ₄		35.0	48.8	62.5	64.2
1N H ₂ SO ₄ + 0		44.1	59.1	69.9	73.4
1N H ₂ SO ₄ + 2N Na ₂ SO ₄		16.2	30.6	43.2	48.9
1N HCl + 0		59.0	72.0	80.4	86.0
1N HCl + 2N MgSO ₄		35.4	50.2	62.0	67.7
1N HCl + 0		60.7	74.2	81.7	87.0
1N HCl + 2N CuSO ₄		47.3	60.8	69.9	74.3

(3) Time intervals for fixed stages of hydrolysis

Solution	Percent hydrolysis						
	35	40	45	50	60	65	70
	Time--in minutes						
1N HCl + 0		73	108	150	261		504
1N HCl + 1N Na ₂ SO ₄		144	192	243	414		837
1N HCl + 1N K ₂ SO ₄		144	192	243	414		837
1N HCl + 3N Al ₂ (SO ₄) ₃		195	246	303	508		999
1N HCl + 0	71	99		171	288		531
1N HCl + 1N Al ₂ (SO ₄) ₃	111	147		246			
1N HCl + 2N Al ₂ (SO ₄) ₃	126	168		270	480		881

TABLE XVIII (Continued)

Solution	Percent hydrolysis						
	35	40	45	50	60	65	70
	Time--in minutes						
1N HCl + 0					282	399	540
1N HCl + 1N MgSO ₄					456	612	801
1N HCl + 2N MgSO ₄					756	945	1126
1N HCl + 2N Na ₂ SO ₄					1035		
1N H ₂ SO ₄ + 0					630	816	1080
1N HCl + 0					315	417	
1N HCl + 2N MgSO ₄					1035	1542	
1N HCl + 0					291	378	480
1N HCl + 2N CuSO ₄					576	810	1125

(4) Velocity constants for fixed stages of hydrolysis

Solution	K x 10 ²						
1N HCl + 0	0.6990	.5540	.462	0.351			0.239
1N HCl + 1N Na ₂ SO ₄	.354	.311	.285	.221			.144
1N HCl + 1N K ₂ SO ₄	.354	.311	.285	.221			.144
1N HCl + 2N Al ₂ (SO ₄) ₃	.261	.243	.229	.184			.120
1N HCl + 0	0.615	.515	.405	.318			.227
1N HCl + 1N Al ₂ (SO ₄) ₃	.388	.347	.282				
1N HCl + 2N Al ₂ (SO ₄) ₃	.343	.303	.256	.191			.137
1N HCl + 0				.325	.263		.223
1N HCl + 1N MgSO ₄				.201	.171		.150
1N HCl + 2N MgSO ₄				.121	.111		.102
1N HCl + 2N Na ₂ SO ₄				.089			
1N H ₂ SO ₄ + 0				.145	.129		.111
1N HCl + 0				.291	.251		
1N HCl + 2N MgSO ₄				.089	.068		
1N HCl + 0				.315	.278		.251
1N HCl + 2N CuSO ₄				.159	.130		.107

(5) Percent decrease in K

1N HCl + 1N Na ₂ SO ₄		49.4	43.9	38.4	37.1		39.7
1N HCl + 1N K ₂ SO ₄		49.4	43.9	38.4	37.1		39.7
1N HCl + 3N Al ₂ (SO ₄) ₃		62.7	56.2	50.5	47.5		49.8
1N HCl + 1N Al ₂ (SO ₄) ₃	36.9	32.6		30.4			
1N HCl + 2N Al ₂ (SO ₄) ₃	44.2	41.2		36.8	40.0		39.6
1N HCl + 1N MgSO ₄					38.2	34.9	31.7
1N HCl + 2N MgSO ₄					62.8	57.9	54.3
1N HCl + 2N Na ₂ SO ₄					72.6		

TABLE XVIII (Continued)

Solution	Percent hydrolysis						
	35	40	45	50	60	65	70
1N H_2SO_4					55.3	51.0	50.3
1N HCl + 2N $MgSO_4$					70.2	73.0	
1N HCl + 2N $CuSO_4$					49.6	53.2	42.7

(6) Average decrease in K--in percent

1N HCl + 1N Na_2SO_4	41.7
1N HCl + 2N Na_2SO_4	72.6
1N HCl + 1N K_2SO_4	41.7
1N HCl + 1N $MgSO_4$	34.9
1N HCl + 2N $MgSO_4$	58.3
1N HCl + 4N $MgSO_4$	71.6
1N HCl + 1N $Al_2(SO_4)_3$	33.3
1N HCl + 2N $Al_2(SO_4)_3$	40.4
1N HCl + 3N $Al_2(SO_4)_3$	53.3
1N HCl + 2N $CuSO_4$	48.5
1N H_2SO_4	52.2

TABLE XIX

EFFECT OF VARIOUS SULFATES ON a_H OF N HCl

AND N H_2SO_4

Solution	pH	a_H	Percent decrease
1N HCl + 0	+0.04	0.91	-
1N HCl + 1N Na_2SO_4	.12	.75	17.6
1N HCl + 2N Na_2SO_4	.22	.60	34.0
1N HCl + 1N $MgSO_4$	.07	.85	6.6
1N HCl + 2N $MgSO_4$	.06	.87	4.4
1N HCl + 4N $MgSO_4$	.01	.98	7.7 ^a
1N HCl + 3N $Al_2(SO_4)_3$	.00	1.00	9.9 ^a
1N HCl + 0	+0.02	0.96	-
1N HCl + 1N $Al_2(SO_4)_3$	.07	.85	11.5
1N HCl + 2N $Al_2(SO_4)_3$	.02	.96	0.0
1N H_2SO_4 + 0	+0.34	0.46	-
1N H_2SO_4 + 2N Na_2SO_4	.61	.25	45.7
1N H_2SO_4 + 2N $MgSO_4$	.40	.40	13.0
1N H_2SO_4 + 2N $Al_2(SO_4)_3$	.34	.46	0.0

^aIncrease

In several cases only a few figures were available for obtaining the averaged results of the decreases in the velocity constant, but observation of the values for $\text{NH}_2\text{-N}$ shows that these results are in accord with what is to be expected. The effect of $2\text{N Na}_2\text{SO}_4$ on $1\text{N H}_2\text{SO}_4$ has not been included in any of the tables after that showing the percent completion of hydrolysis at a definite time interval, as it was desired only to show that the salt will retard the action of H_2SO_4 as well as that of HCl .

The sulfates invariably depress the rate of hydrolysis, and the amount of depression seems to be a function of both the nature of the salt and its concentration, the higher the concentration the greater being the retardation. This is true despite the fact that several of these acid-solutions, as shown in the above table, actually have a higher a_{H} than the HCl alone. These activities were measured at 25° in the absence of gelatin. The rates of hydrolysis of the $2\text{N Na}_2\text{SO}_4$ and 4N MgSO_4 solutions are considerably lower than that of the $\text{N H}_2\text{SO}_4$, although the a_{H} of the acid is much lower than that of the two acid-salt solutions. The hydrolysis rate of this H_2SO_4 solution has been compared with that of the N HCl in the same manner as that used for the acid-salt solutions.

The possible explanations for this salt effect are the same as those advanced previously; namely, the a_{H} of the solution may be greatly altered by the gelatin, or there may be an irregular temperature coefficient of a_{H} in the presence of the salts.

The following experiments were carried out in order to demonstrate whether the first explanation is significant. The pH was taken of solutions containing $1\text{N Na}_2\text{SO}_4$ and $3\text{N Al}_2(\text{SO}_4)_3$

respectively (in the presence of 1N HCl), gelatin added as usual, and the pH taken again after 20 hours heating over a boiling water bath. The results were compared with that obtained with an HCl solution containing no salt.

TABLE XX
EFFECT OF GELATIN ON a_H OF HCl-SULFATE SOLUTIONS

Solution	Before hydrolysis (no gelatin)		After hydrolysis (gelatin present)	
	pH	a_H	pH	a_H
1N HCl + 0	+0.02	0.96	+0.02	0.96
1N HCl + 1N Na_2SO_4	+0.11	.78	+0.15	.71
1N HCl + 3N $\text{Al}_2(\text{SO}_4)_3$	-0.02	1.05	+0.05	.89

The changes in the salt solutions are not very large, and in the case of the $\text{Al}_2(\text{SO}_4)_3$ it is hardly enough to explain why this solution should hydrolyze at a rate practically the same as that of 1N H_2SO_4 which has a much lower a_H . The tendency of salts to shift the pH of acid-gelatin solutions has been reported by Simms⁹ who has worked with solutions very much weaker than these.

When solutions of Na_2SO_4 and MgSO_4 were made up so that the concentration of salt was 1N, of HCl was 1N, and of gelatin was 1%, and such solutions compared with similar ones made up in the absence of gelatin, it was found that the gelatin solutions were both 0.02 pH more alkaline. Thus, under these conditions of concentration the protein effects the pH of the acid-salt solutions to the same extent, and this effect is so slight that it can hardly be the cause of the observed discrepancies between hydrolysis rates and activities.

⁹ H. S. Simms, J. Gen. Physiol., 11 (1927-1928), 613.

Since 4N MgSO_4 precipitated the gelatin, the foregoing experiment with this concentration of salt was modified in this manner. 0.5 g. of gelatin was added to 50 cc. of a solution of 1N HCl + 4N MgSO_4 , the solution kept in the icebox over night until the gelatin had swollen as much as it would, then heated over a boiling water bath for several hours with shaking until solution was complete. After cooling to 25° the pH was taken and compared with a similar acid-salt solution which contained no gelatin.

TABLE XXI
EFFECT OF GELATIN ON a_H OF HCl-SULFATE SOLUTIONS

Solution	Before hydrolysis (no gelatin)		After hydrolysis (gelatin present)	
	pH	a_H	pH	a_H
1N HCl + 0	+0.02	0.96		
1N HCl + 4N MgSO_4	- .01	1.02	+0.05	0.89

The a_H of 4N MgSO_4 is affected by the protein to practically the same extent as is 3N $\text{Al}_2(\text{SO}_4)_3$; however, the magnesium salt has a markedly greater depressing action on the hydrolysis rate; on the other hand, with H_2SO_4 alone, the hydrolysis rate is the same or greater, while the a_H is much lower than that of HCl in the presence of these salts.

As has been pointed out several times, these activities have been measured at 25° whereas the hydrolysis was carried out at 100°. In order to learn whether the temperature coefficients of the acid-sulfate solutions vary from that of the HCl, the activities of several of these solutions were measured at different temperatures in the same manner as that previously described for NaCl. The results are given in the following table.

TABLE XXII
TEMPERATURE COEFFICIENTS OF HCl AND HCl-SULFATE
SOLUTIONS

Solution	Temperature					
	25		36.5		47	
	a_H	Percent Decrease in a_H	a_H	Percent Decrease in a_H	a_H	Percent Decrease in a_H
1N HCl + 0	0.89	-	0.95	-	1.29	-
1N HCl + 2N Na_2SO_4	.55	37	.48	50	.46	63
1N HCl + 2N $MgSO_4$	.83	7	.58	39	.71	45
1N HCl + 4N $MgSO_4$	1.02	15 ^a	.74	22	.69	46
1N HCl + 2N $Al_2(SO_4)_3$	.91	2 ^a	.89	6	.83	36

^aIncrease

The results in the above table indicate that irregular temperature coefficients of a_H are responsible for the discrepancies observed between the effect of the salts on the a_H of HCl as measured at 25° and the effect on the hydrolysis rates. The respective coefficients obtained for 2N and 4N $MgSO_4$, even in the limited range used here, point to the fact that the temperature coefficient drops with increasing sulfate concentration. In the case of the magnesium and aluminum salts at least, this is probably the explanation for the regular drop in hydrolysis rate with increasing sulfate concentration irrespective of the effect of the salt on the a_H of the acid at 25°. Since increasing concentrations of Na_2SO_4 cause a drop in a_H even at 25° it is not possible to make such a statement about this salt in the absence of additional data.

As the sulfates had such a marked effect on the speed of hydrolysis, it became of interest to learn whether complete hydrolysis is possible in their presence. Samples of gelatin were hydrolyzed in the presence of the salts indicated below,

by boiling the solutions over hot plates under reflux condensers until the amino nitrogen became constant. A second lot of Eastman's gelatin was used in this run, differing from the first only in that its moisture content was 11.3% instead of 10.7%

TABLE XXIII
COMPLETE HYDROLYSIS OF GELATIN IN THE PRESENCE
OF SULFATES

Solution	Hours boiling						
	125	150	175	190	225	240	275
	Percent $\text{NH}_2\text{-N}$						
1N HCl + 0	11.80	11.70		11.65			
1N HCl + 0	11.85	11.65		11.65			
1N HCl + 2N Na_2SO_4	11.05	11.25		11.15		11.20	
1N HCl + 2N MgSO_4	11.55	11.40		11.55			
1N H_2SO_4 + 0	11.45		11.65		11.85		
1N H_2SO_4 + 0	11.45		11.80		11.65		
1N H_2SO_4 + 2N Na_2SO_4			10.20		10.60		
1N H_2SO_4 + 2N MgSO_4			11.20		11.45		11.45
1N H_2SO_4 + 2N $\text{Al}_2(\text{SO}_4)_3$			11.25		11.35		

In every case in which salts of H_2SO_4 were used there was inability to obtain the full amount of $\text{NH}_2\text{-N}$, although 1N H_2SO_4 alone hydrolyzed the gelatin completely if enough time was allowed. The values for the HCl and H_2SO_4 agree exactly on duplicate determinations, the duplicates for each being only 0.05% apart; this refers to the highest values obtained for each. In the absence of more complete data on temperature coefficients of a_H it is not possible to state whether the above results with sulfates are due to lessened a_H at high temperatures or whether they are due to actual chemical reaction between these salts and the protein or its hydrolysis products. The solution

of this problem would be in experiments in which pH measurement and hydrolysis are carried out at the same temperature.

Hydrolysis of gelatin solutions of same pH
in sealed tubes

In all the previous work the method used for studying the action of salts on the hydrolysis of the protein has been that of starting with solutions of the same acid concentration but of different a_H . A second method is that of hydrolyzing solutions of different acid concentration but of the same a_H . If the a_H is the determining factor in speed of hydrolysis, and if acid-salt solutions have a lower temperature coefficient of a_H than does the acid alone, then the salts should invariably depress the hydrolysis rate.

The general procedure was to bring the various solutions to the same pH at room temperature in the presence of gelatin, the samples were then hydrolyzed in a boiling water bath for a definite time interval in sealed 8 inch Pyrex test tubes, the tubes opened, and 5 cc. samples removed and diluted to volume in a 10 cc. volumetric flask. Two cc. of this diluted solution were used for analysis for NH_2-N .

Bringing the solutions to the same pH was accomplished in the following manner. Various accurately measured volumes of 2.146N HCl were diluted to 100 cc. in the absence or presence of the different salts, 5 cc. removed and added to 5 cc. of a 2% gelatin solution. The pH was then determined by the hydrogen electrode. According to the pH observed, the volumes of HCl were varied until the same pH was realized in all solu-

tions. The gelatin, measured accurately by volume, was also weighed after addition to the acid-salt solution, and the error of volumetric measurement was found to be not more than 0.03 cc. Tubes containing gelatin and HCl alone were always run as controls.

The necessity for bringing the acid-salt solutions to the same pH in the presence of gelatin rather than in its absence is demonstrated by the following experiment.

TABLE XXIV
EFFECT OF GELATIN ON pH OF ACID AND ACID-SALT
SOLUTIONS

1. 70 cc. 2N HCl diluted to 100 cc.		
5 cc. of foregoing solution + 5 cc water		pH +0.22
5 cc. of foregoing solution + 5 cc 2% gelatin		pH +0.23
2. 50 cc. 2N HCl diluted to 100 cc.--containing 1N NaCl		
5 cc. of foregoing solution + 5 cc. water		pH +0.28
5 cc. of foregoing solution + 5 cc. 2% gelatin		pH +0.30
(Final concentration of NaCl = 0.5N)		
3. 73 cc. 2N HCl diluted to 100 cc.--containing 1N Na ₂ SO ₄		
5 cc. of foregoing solution + 5 cc. water		pH +0.23
5 cc. of foregoing solution + 5 cc. 2% gelatin		pH +0.28
(Final concentration of Na ₂ SO ₄ = 0.5N)		

Under these conditions of acid, salt, and protein concentration the gelatin has very little effect on the acid or acid-NaCl solution, distinctly more on the acid-Na₂SO₄.

In the following tables, along with the figures for NH₂-N, are given the volumes of 2N HCl necessary to obtain the given pH in the presence or absence of the salt. The salt concentrations are the final ones after addition of the gelatin to the acid-salt

solutions. In all cases the pH was obtained by taking equal volumes of the acid or acid-salt solution and 2% gelatin. Similarly, 5 cc. of the acid or acid-salt solution and 5 cc. of 2% gelatin were placed in 8 inch Pyrex test tubes, the tubes sealed, and heated over a boiling water bath as indicated for each experiment.

TABLE XXV
HYDROLYSIS OF GELATIN SOLUTIONS OF SAME pH

(1) Volumes of 2N HCl necessary for dilution		
Volume	pH	a _H
62 cc. 2N HCl diluted to 100 cc.	+0.29	0.51
52 cc. 2N HCl diluted to 100 cc. (NaCl = 0.5N)	.29	.51
84 cc. 2N HCl diluted to 100 cc. (Na ₂ SO ₄ = 1N)	.27	.54
(2) Percent NH ₂ -N (10 hours heating)		
HCl + 0	8.30%	
HCl + 0	8.30	
HCl + 0.5N NaCl	8.05	
HCl + 1N Na ₂ SO ₄	7.80	
(3) pH of solutions after hydrolysis		
HCl + 0	+0.31	
HCl + 0	.31	
HCl + 0.5N NaCl	.32	
HCl + 1N Na ₂ SO ₄	.30	

The pH has changed a little as a result of the hydrolysis, but practically the same in all cases. The decreases caused by the salts are all tabulated together at the end of all the experiments of this type.

The results of the next two runs are tabulated below.

TABLE XXVI
HYDROLYSIS OF GELATIN SOLUTIONS OF SAME pH

(1) Volumes of 2N HCl necessary for dilution					
Volume				pH	a _H
62	cc.	2N HCl	diluted to 100 cc.	+0.29	0.51
40	cc.	2N HCl	diluted to 100 cc. (NaCl = 1N)	.30	.50
76	cc.	2N HCl	diluted to 100 cc. (MgSO ₄ = 1N)	.29	.51
32.5	cc.	2N HCl	diluted to 100 cc. (H ₂ SO ₄ = 1N)	.30	.50
20	cc.	20% HCl	diluted to 100 cc. (H ₃ PO ₄ = .33 M)	.29	.51

(2) Percent NH ₂ -N (10 hours heating)			
HCl	+	0	8.00%
HCl	+	0	8.00
HCl	+	1N NaCl	7.50
HCl	+	1N MgSO ₄	7.50
HCl	+	1N H ₂ SO ₄	8.00
HCl	+	0.33M H ₃ PO ₄	8.05

This run included H₂SO₄ in order to learn whether the retardation by the sulfates was also characteristic of the acid. In studying phosphates it was necessary to use the free phosphoric acid instead of its salts because of the buffer action of the latter.

TABLE XXVII
HYDROLYSIS OF GELATIN SOLUTIONS OF SAME pH

(1) Volumes of 2N HCl necessary for dilution					
Volume				pH	a _H
62	cc.	2N HCl	diluted to 100 cc.	+0.27	0.54
73	cc.	2N HCl	diluted to 100 cc. (Na ₂ SO ₄ = 0.5N)	.28	.53
57	cc.	2N HCl	diluted to 100 cc. (CaCl ₂ = 0.5N)	.28	.53
43	cc.	2N HCl	diluted to 100 cc. (CaCl ₂ = 1N)	.28	.53
28	cc.	2N HCl	diluted to 100 cc. (CaCl ₂ = 2N)	.27	.54
70	cc.	2N HCl	diluted to 100 cc. (Al ₂ (SO ₄) ₃ = 0.5N)	.27	.54

(2) Percent NH ₂ -N (8 hours heating)			
HCl	+	0	7.65%
HCl	+	0	7.65
HCl	+	0.5N Na ₂ SO ₄	7.60

TABLE XXVII (Continued)

HCl + 0.5N CaCl_2	7.65%
HCl + 1N CaCl_2	7.55
HCl + 2N CaCl_2	6.75
HCl + 0.5N $\text{Al}_2(\text{SO}_4)_3$	7.55

When it was attempted to take the pH of solutions whose final concentrations were 2N in NaCl, 2N in MgSO_4 , and 1.5N in $\text{Al}_2(\text{SO}_4)_3$, the gelatin was precipitated. It was felt that the readings were not reliable in these cases, and the solutions were not used.

In the following table are summarized the results of the above-mentioned experiments, the decreases being expressed in percent of $\text{NH}_2\text{-N}$ of the total present after the specified period of heating. Allowance was made for 0.55% of $\text{NH}_2\text{-N}$ present before hydrolysis. The solution containing HCl alone has been used as the basis for the calculation.

TABLE XXVIII

PERCENT DECREASE IN $\text{NH}_2\text{-N}$ CAUSED BY SALTS

0.5N NaCl	3.2%
1N NaCl	6.7
0.5N Na_2SO_4	0.7
1N Na_2SO_4	6.5
0.5N CaCl_2	0.0
1N CaCl_2	1.4
2N CaCl_2	12.7
1N MgSO_4	6.7
0.5N $\text{Al}_2(\text{SO}_4)_3$	1.4

Normal H_2SO_4 and 0.33M H_3PO_4 have no effect on the speed of hydrolysis, the difference of 0.05% $\text{NH}_2\text{-N}$ in the case of the latter being no greater than the experimental error of the analytical method.

In the case of the salts, the foregoing figures show that with NaCl , Na_2SO_4 , and CaCl_2 , under these particular conditions the rate of hydrolysis decreases as the salt concentration increases. If we assume that the hydrolysis rate is determined by the a_{H} at the temperature of hydrolysis, then each salt appears to have its own characteristic temperature coefficient, the coefficient falls with rising concentration of the salt, and it is not necessarily a regular function of the concentration. These conclusions are the same as those which can be drawn from the results presented in the first part of this work.

Effect of small changes in pH on
hydrolysis rate

To calculate the effect of small changes in pH on the hydrolysis rate, the differences in $\text{NH}_2\text{-N}$ between hydrolysis by 1N and 2N HCl can be compared. The greatest difference occurs at 0.5 hours and amounts to 2.15% nitrogen. The pH difference between the acids is 0.38, and assuming that in this range of pH the ratio of change of hydrolysis rate to change of pH is uniform, a variation of 0.02 pH will cause a difference in $\text{NH}_2\text{-N}$ of 0.11%. This value decreases only slightly through 20 hours heating, dropping to 0.074%; at this point the hydrolysis by 1N HCl is about 80% complete. A change of 0.02 pH corresponds to an average change of 0.056 in normality of the acid within this range.

These figures can be used to determine the effect of concentration on the hydrolysis rate of the protein. After a solu-

tion, containing originally 50 cc., has been sampled four times, there remain 30 cc. If the maximum loss by evaporation during any one interval of heating is 1.5 cc., then the normality of the acid during the period of heating following the fourth sampling will change by 0.056. This change is the greatest that would take place under the conditions of hydrolysis as used here, and would cause an increase in $\text{NH}_2\text{-N}$ of about 0.10% except in the last stages, during which the increase is less. However, since the loss of water certainly did not all occur at the beginning but most logically is to be thought of as taking place gradually during the entire interval, then the increase is 0.05% instead of 0.10%, and 0.05% is the experimental error of the Van Slyke determination in this work.

That this calculation is valid is shown by the following experiment in which the acid used is of about the strength of normal in one case and a little stronger in the second case. These samples were heated for 6 hours in sealed tubes over a boiling water bath, the solutions being prepared in the same manner as that previously described in the work on sealed tubes.

TABLE XXIX
EFFECT OF SMALL CHANGES IN pH ON HYDROLYSIS
OF GELATIN

Solution	Percent $\text{NH}_2\text{-N}$	Percent increase in $\text{NH}_2\text{-N}$
HCl-- pH +0.05	8.35%	-
HCl-- pH +0.05	8.30	-
HCl-- pH -0.03	8.65	4.1
HCl-- pH -0.03	8.60	3.5

Here a difference of 0.08 pH makes a difference of 0.30% $\text{NH}_2\text{-N}$, and thus a change of 0.02 pH would cause a difference of

0.075% $\text{NH}_2\text{-N}$.

Action of salts on nitrous acid

It was observed during the analyses for amino nitrogen that some of the salts gave an excessive amount of gas. The effects of the salts on the HNO_2 was then investigated, and it was found that 0.5N and 1N HCl , all the iron and aluminum salts used, and the iodides in 1.5N and 2N but not in 0.25N solutions, gave high blanks. The other salts used in this work did not raise the blanks. The values in the table indicate the increases observed with the compounds mentioned above.

TABLE XXX
ACTION OF SALTS ON NITROUS ACID

Solution	Vol. N_2	Bar.	Temp.	Mgms. $\text{NH}_2\text{-N}$
H_2O	0.11	743.0	25.0	0.060
H_2O	.11	743.0	25.0	.060
0.5N HCl	.125	743.0	26.0	.067
0.5N HCl	.12	743.0	26.0	.065
1N HCl	.12	743.0	25.5	.065
1N HCl	.12	743.0	25.5	.065
0.5N HCl + 1.5N Al_2Cl_6	.145	743.0	24.0	.079
0.5N HCl + 1.5N Al_2Cl_6	.155	743.0	25.0	.083
0.5N HCl + 1.5N $\text{Al}_2(\text{SO}_4)_3$	.16	742.0	26.5	.086
0.5N HCl + 1.5N $\text{Al}_2(\text{SO}_4)_3$	.16	742.0	26.5	.086
0.5N HCl + 1.5N $\text{Al}(\text{NO}_3)_3$	.165	742.0	26.5	.089
0.5N HCl + 1.5N $\text{Al}(\text{NO}_3)_3$	.17	742.0	26.5	.091
H_2O	.115	740.5	29.0	.061
H_2O	.115	740.5	29.0	.061
0.5N HCl + 0.75N Al_2Cl_6	.13	740.5	29.0	.069
0.5N HCl + 0.75N Al_2Cl_6	.13	740.5	29.0	.069
0.5N HCl + 0.25N $\text{Al}_2(\text{SO}_4)_3$	.145	749.5	24.5	.080
0.5N HCl + 0.25N $\text{Al}_2(\text{SO}_4)_3$	.145	749.5	24.5	.080
0.5N HCl + 0.5N $\text{Al}_2(\text{SO}_4)_3$	.16	740.5	29.0	.085
0.5N HCl + 0.5N $\text{Al}_2(\text{SO}_4)_3$	.155	740.5	29.0	.082
0.5N HCl + 1N $\text{Al}_2(\text{SO}_4)_3$	.165	740.5	29.0	.087
0.5N HCl + 1N $\text{Al}_2(\text{SO}_4)_3$	.16	740.5	29.0	.085

TABLE XXX (Continued)

Solution	Vol. N ₂	Bar.	Temp.	Mgms. NH ₂ -N
H ₂ O	0.10	752.0	25.5	0.055
H ₂ O	.10	752.0	25.5	.055
0.5N HCl + 0.375N FeCl ₃	.105	752.0	25.5	.058
0.5N HCl + 0.375N FeCl ₃	.115	752.0	25.5	.063
0.5N HCl + 0.75 N FeCl ₃	.11	752.0	25.5	.060
0.5N HCl + 1.5 N FeCl ₃	.18	751.0	25.5	.098
0.5N HCl + 6 N FeCl ₃	.35	750.5	26.0	.191
0.5N HCl + 9 N FeCl ₃	.37	750.5	26.0	.202
H ₂ O	.115	744.5	25.0	.063
H ₂ O	.11	744.5	25.0	.060
0.5N HCl + 3N Fe ₂ (SO ₄) ₃	.195	744.5	25.0	.106
0.5N HCl + 3N Fe ₂ (SO ₄) ₃	.195	744.5	25.0	.106

In concentrations of 0.75N or less the FeCl₃ does not increase the blank. Since higher concentrations of the salt generated great volumes of gas it was felt that perhaps so much HNO₂ was decomposed in this manner that there was not enough left to react quantitatively with the amino groups of the protein hydrolysates. To test this, FeCl₃ was added to a solution of glutamic acid hydrochloride of such strength that a 2 cc. sample gave about as much nitrogen as the protein solutions conventionally used. No HCl was added to these samples. The results are recorded below.

TABLE XXXI

AMINO NITROGEN CONTENT OF GLUTAMIC ACID
HYDROCHLORIDE

(1) In the presence of FeCl₃

Solution	Vol. N ₂	Bar.	Temp.	Mgms. NH ₂ -N
H ₂ O	0.105	750.5	24.5	0.058
Glutamic acid--HCl	1.81	750.5	24.5	.994
Glutamic acid--HCl + 1.5N FeCl ₃	1.885	750.5	25.0	1.033

TABLE XXXI (Continued)

Solution	Vol. N ₂	Bar.	Temp.	Mgms. NH ₂ -N
Glutamic acid--HCl + 6N FeCl ₃	2.04	750.5	25.5	1.117
Glutamic acid--HCl + 9N FeCl ₃	1.88	748.0	26.0	1.021

By applying the proper blanks for the FeCl₃ solutions, it can be seen from the foregoing figures that the excess nitrogen is returned quantitatively with 1.5N FeCl₃, almost so with the 6N salt, but that it is quite low when the salt concentration is 9N. So much nitrite is decomposed by the 9N FeCl₃ that not enough is left to react quantitatively with the amino acid.

KI solutions were used in the same manner.

TABLE XXXI (Continued)

(2) In the presence of KI

Solution	Vol. N ₂	Bar.	Temp.	Mgms. NH ₂ -N
H ₂ O	0.115	748.0	23.5	0.063
2N KI	.15	748.0	23.5	.083
Glutamic acid-HCl no. 1	1.845	739.5	26.5	.987
Glutamic acid-HCl no. 1 + 2N KI	1.87	739.5	26.0	1.003
Glutamic acid-HCl no. 2	2.30	748.0	25.0	1.256
Glutamic acid-HCl no. 2 + 2N KI	2.285	748.0	25.0	1.248

In the case of the first amino acid solution, the excess nitrogen is recovered practically completely. With the second solution, approximately 25% stronger in glutamic acid, there is an appreciable difference between the observed value and that which had been calculated by reason of the high blank for the iodide. Here again there seems to have been so much destruction of the nitrite by the iodide that not enough is left for a quantitative reaction with the stronger solution of the amino acid.

All results in the above tables have been recorded as corrected milligrams of amino nitrogen. Two cc. samples were used throughout.

Discussion of accuracy of calculations

In determining the accuracy to ascribe to calculations of changes in the rate of hydrolysis, two factors must be considered--the ability to obtain check results with duplicates and the amount of $\text{NH}_2\text{-N}$. 0.20% of amino nitrogen is the greatest deviation to be observed between duplicate determinations, and usually the difference is not more than half this. Thus, when the total $\text{NH}_2\text{-N}$ is only 2%, the duplicates themselves might show a difference of 10% of the total in the hydrolysis rate, and no deviation less than this is of significance. When the total $\text{NH}_2\text{-N}$ is 7%, the significant difference is 3% of the total and so forth.

With the sealed tubes the duplicates check within 0.10% of nitrogen in every case except one, in which the difference reached 0.15%. A deviation of 0.10% amounts to 1.3% of the total when the $\text{NH}_2\text{-N}$ is 7.5%. The error of sampling is estimated at not more than 0.60% of the total. Since the total $\text{NH}_2\text{-N}$ is practically the same in all these cases, one calculation can be made to indicate the significant deviation, which is $1.3 + 0.6$, or at most, 2% of the total.

SUMMARY

1. A study has been made of the effect of various salts on the rate of hydrolysis of gelatin by 1N HCl at 100°.

For mathematical formulation of the relative rates of hydrolysis, the method was used of calculating the first order velocity constants at fixed degrees of hydrolysis. In general the addition of halides accelerates the hydrolysis as compared with the acid alone, whereas the sulfates retard the hydrolysis.

2. When attempts were made to correlate the changes in hydrolysis rate effected by the addition of salts with the effects of the salts on the a_H measured at room temperature, wide discrepancies appeared. Not only were there quantitative differences between the relative changes in the two values, but in some cases increases in a_H , measured at room temperature, were accompanied by decreases in the hydrolysis rate. These discrepancies in the action of the salts are explained by the fact that the temperature coefficients of a_H of the acid-salt solutions are lower than that of the acid alone. Measurements of a_H at 25°, 36.5°, and 47°, are given to support this view.

3. Each salt seems to have its characteristic temperature coefficient of a_H , and the coefficient is not necessarily a regular function of the salt concentration.

4. Although salts of H_2SO_4 retard the hydrolysis of gelatin by HCl , H_2SO_4 itself does not.

5. It was found impossible to obtain complete hydrolysis by either 1N HCl or 1N H_2SO_4 in the presence of several 2N sulfate solutions, although each acid alone is capable of causing complete hydrolysis. It is not known whether this inhibition is also due to lowered temperature coefficients of a_H , or to some other specific effect of the salt.

6. No Hofmeister series for the action of alkali halides on the acid hydrolysis of proteins could be demonstrated.

